Eight types of stem cells in the life cycle of the moss Physcomitrella patens

doi: 10.1016/j.pbi.2013.10.007

Eight types of stem cells in the life cycle of the moss *Physcomitrella patens*

Rumiko Kofuji[1] and Mitsuyasu Hasebe[2,3]

Address

[1] Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa 920-1192, Japan

[2] National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki 444-8585, Japan

[3] Department of Basic Biology, Graduate School of Advanced Studies, Okazaki 444-8585, Japan

Corresponding author:

Prof. Mitsuyasu Hasebe

National Institute for Basic Biology

38 Nishigonaka, Myodaiji-cho

Okazaki 444-8585

Japan

e-mail: mhasebe@nibb.ac.jp

Phone: +81-564-55-7546

Highlights

1. At least eight types of stem cells are recognized in the life cycle of the moss *Physcomitrella patens.*
2. We reviewed gene regulatory networks to form each type of stem cells.
3. Class 2 KNOX and polycomb repression complex 2 genes inhibit to make stem cells of the other generation.
4. Differentiated *Physcomitrella patens* cells are easily reprogrammed to stem cells.

Abstract

Stem cells self-renew and produce cells that differentiate to become the source of the plant body. The moss *Physcomitrella patens* forms eight types of stem cells during its life cycle and serves as a useful model in which to explore the evolution of such cells. The common ancestor of land plants is inferred to have been haplontic and to have formed stem cells only in the gametophyte generation. A single stem cell would have been maintained in the ancestral gametophyte meristem, as occurs in extant basal land plants. During land plant evolution, stem cells diverged in the gametophyte generation to form different types of body parts, including the protonema and rhizoid filaments, leafy-shoot and thalloid gametophores, and gametangia formed in moss. A simplex meristem with a single stem cell was acquired in the sporophyte generation early in land plant evolution. Subsequently, sporophyte stem cells became multiple in the meristem and were elaborated further in seed plant lineages, although the evolutionary origin of niche cells, which maintain stem cells is unknown. Comparisons of gene regulatory networks are expected to give insights into the general mechanisms of stem cell formation and maintenance in land plants and provide information about their evolution. *Physcomitrella patens* develops at least seven types of simplex meristem in the gametophyte and at least one type in the sporophyte generation and is a good material for regulatory network comparisons. In this review, we summarize recently revealed molecular mechanisms of stem cell initiation and maintenance in the moss.

Diversity of stem cells in land plants

Stem cells have the ability to self-renew and to form differentiated cells, and several types of stem cells are formed during development and growth [1,2]. Since stem cells are the sources of body parts, their appropriate regulation is necessary in order to generate the body plan successfully. The morphological and anatomical organization of stem cells varies among land plants [3] (Table 1), and comparisons of their regulatory systems in different land plant lineages should be useful in deducing the evolution of body plans as well as understanding the commonalities and differences in stem cell regulatory systems.

Stem cells of flowering plants are formed in the diploid generation and retained in the shoot meristem, root meristem, and cambium. In shoot and root meristems, stem cells are accompanied by niche cells such as those in metazoa to function in the maintenance of stem cells. The haploid bodies of flowering plants have been reduced to a small number of cells and do not contain stem cells. Gymnosperm shoots and roots also have multiplex meristems; a type of meristem with multiple stem cells [3, 4], although whether they contain niche cells is not known. Haploid gymnosperm bodies are multicellular but do not include stem cells. On the other hand, non-seed land plants, with a few exceptions in the Lycopodiaceae and Isoetaceae, retain the simplex meristem with a single apical stem cell in both the haploid and diploid generations [3]. Niche cells have not been well-characterizes experomentally in either generation of these plants.

Sporophyte stem cells of monilophytes and lycopods retain indeterminate cell division activity, but gametophyte stem cells are maintained for only a relatively short period in most species. In bryophytes, the longevity of diploid stem cells is limited, resulting in a diploid body with a semi-parasitic habit on the dominant haploid body, where several types of indeterminate stem cells are formed. Although most non-seed plants form simplex meristems, the nature of stem cells is variable in terms of the number of division planes and differentiated cells they produce. Even within the haploid generation of a given species, several types of stem cells are formed.

In this review, we will summarize the molecular mechanisms responsible for forming different types of stem cells in both generations of the moss *Physcomitrella patens* (Physcomitrella). In recent years, almost all stem cell research on non-flowering plants has been performed in Physcomitrella because of the availability of a facile gene

analysis system [5,6] and genome resources [7,8], as well as its simple body architecture that facilitates live and observations and *in vivo* analysis of stem cells (http://moss.nibb.ac.jp).

Stem cells in the Physcomitrella haploid generation

In Physcomitrella, seven recognized types of stem cells form different haploid tissues and organs (Box1 and Figure 1). The first division of a spore forms a chloronema apical stem cell that undergoes tip growth [9] and continuously produces chloronema cells to form a filamentous body: the chloronema. Chloronema cells that are not to next to the apical stem cell usually form chloronema side branch initials that become secondary chloronema apical stem cells before dividing. It is not yet known when a side branch initial cell is fated as a stem cell. The secondary stem cells grow in the same manner as the primary one, resulting in a branched filamentous chloronema body.

A haploid generation similarly arises from a single cell during the regeneration of protoplasts isolated from chloronemata. After the recovery of a cell wall [10], protoplasts commence tip growth and divide asymmetrically, similar to a chloronema apical stem cell. Transcriptome data will be useful for future functional analyses of genes involved in stem cell formation from protoplasts [11].

After several days of cultivation, chloronema apical stem cells transform into caulonema apical stem cells that produce caulonema cells [12]. Both chloronemata and caulonemata are filamentous, and together they are referred to as protonemata. Chloronema cells with round, green chloroplasts are adaptive for photosynthesis, whereas caulonema cells, which are characterized by more rapid growth and spindle-shaped, less-green chloroplasts, are adapted for expansion. The transition from a chloronema to a caulonema apical stem cell is enhanced and retarded by glucose and ammonium ion, respectively, supplemented in the medium, suggesting that the stem cell transition is regulated by the carbon/nitrogen ratio [13] via photosynthesis and metabolic genes including hexokinase [14,15]. Phytochrome and cryptochrome signaling pathways inhibit the chloronema-to-caulonema transition [16,17]. Auxin [18] and the basic helix-loop helix transcription factors ROOT HAIR DEFECTIVE SIX-LIKE1 (PpRSL1) and PpRSL2 [19] as well as diterpenes derived from a gibberellin precursor [20] positively regulate the transition, whereas cytokinin is a

negative regulator [15]. Auxin and cytokinin signaling pathways also interact in Arabidopsis, and auxin signaling pathways involving TIR1-IAA/AUX and the cyclophilin DIAGEOTROPICA are conserved between Physcomitrella and Arabidopsis [21,22]. Comparison of the crosstalk between these two distantly related species should give insights into the general features and evolution of signaling networks in land plants [23].

Caulonema cells form side branch initial cells, of which 87% are fated to become secondary chloronema apical stem cells, 5% become secondary caulonema apical stem cells, 5% form gametophore apical stem cells, and 3% are non-dividing cells [24]. Side branch initial cells fated as gametophore apical stem cells swell with diffuse growth instead of tip growth, and successive cell divisions form a tetrahedral apical stem cell with three oblique cutting faces resulted from cell divisions [25] (Figure 2a). Differently from protonema apical stem cells, the gametophore apical stem cells form a preprophase band at the future cell plate attachment position of the mother cell [26], likely with similar molecular mechanisms to division-plane formation of flowering plants [27]. Development of the haploid shoots, the gametophores, is stereotypic and a gametophore apical cell at the tip continuously produces primordial cells for a stem and leaves [25].

Several loss-of-function mutants show decreased numbers of gametophores [13,28]. AP2-type transcription factor genes orthologous to Arabidopsis *AINTEGUMENTA*, *PLETHORA*, *BABY BOOM*, and their paralogs (*APB*s) were found to be indispensable for gametophore apical stem cell formation in the side branch initial cells [29]: the quadruple disruption mutant of all *APB* genes in the genome does not form gametophores. All four APB proteins are continuously expressed in side branch initial cells fated to be gametophore apical stem cells but diminish in side branch initial cells that become chloronema or caulonema apical stem cells. As with Arabidopsis orthologs, *APB* transcripts are positively regulated by auxin. On the other hand, cytokinin is known to enhance gametophore apical stem cell formation [18,30]. Cytokinin negatively regulates PpMIR534a, which in turn negatively regulates the transcript level of *PpBOP1/2*, a positive regulator of gametophore stem cell formation [31]. These findings suggest that an auxin-signaling pathway involving APBs and a cytokinin-signaling pathway involving miR534a and PpBOP1/2 synergistically determine gametophore apical stem cell fate. However, spatiotemporal regulation of

auxin and cytokinin in the side branch initials and their mother caulonema cells are mostly unexplored. In some cases, both secondary caulonema and gametophore apical stem cells are contemporarily formed from the same mother caulonema cell, suggesting that as yet unknown regulatory mechanisms provide local cues for stem cell fate establishment [25].

Gametophore apical stem cells successively produce cells to form leaf apical stem cells (Figure 2b). A leaf apical stem cell has two cutting faces that produce two rows of wedge-shaped cells, whose subsequent cell divisions result in a planar leaf composed mostly of a single cell layer [25] (Figure 2c). No genes that clearly function in leaf apical stem cells have been reported to date.

Rhizoids, which function to acquire nutrients and solutes, are composed of filamentous tissue with a rhizoid apical stem cell at the tip [32]. Rhizoids are similar to protonemata but have brown pigments in the cell wall and immature plastids [33]. Rhizoid apical cells initiate from an epidermal cell of a gametophore stem in a process that is hypothesized to be related to auxin distribution [33]. Auxin regulates *RSL1* and *RSL2* expression in the primordial epidermal cells that give rise to rhizoid apical stem cells and is necessary and sufficient for rhizoid formation [34,35]. To understand the spatiotemporal relationship between auxin amounts and *RSL* induction, visualization of auxin concentrations at the cellular level using auxin sensors [36] will give insights into how auxin is regulated to induce the stem cells.

Egg- and sperm-bearing organs, the archegonia and antheridia respectively, are formed at the tip of gametophores under low temperature and short day conditions [37]. Each reproductive organ is formed from a stem cell (Figure 2d,e), although it is unknown whether gametangia stem cells are derived from gametophore stem cells or initiate *de novo* from differentiated cells. Several archegonia and antheridia are distally formed around the initial archegonium and antheridium, respectively [38], but the origins of their stem cells are not known. Both types of gametangia stem cells have two cutting faces to produce two rows of cells. An antheridium apical stem cell produced six wedge-shape cells subsequently divide periclinally to form outer jacket cells and inner spermatogenous cells [39]. After six rounds of cell division, an antheridial apical cell loses its division activity. An archegonium stem cell produces approximately four wedge-shape cells in two rows and then changes its division plane to form an inner cell that differentiates into an egg cell, a ventral canal cell, and neck canal cells [39].

Physcomitrella sporophyte stem cells

Sperm swims to an archegonium and enters the cavity for fertilization through the opened neck. The first cell division of the resulting zygote is asymmetrical and forms a sporophyte apical stem cell and a basal cell (Figure 2f). *LEAFY* genes, which function as floral homeotic genes in angiosperms, are indispensable in this process [40,41]. The sporophyte apical stem cell has two cutting faces and successively divides approximately 12 times to form two rows of cells [33] (Figure 2f). A component of the polycomb repression complex 2 (PRC2), CURLY LEAF (CLF), negatively regulates the longevity of the sporophyte apical stem cell [42]. CLF protein is detected in most gametophyte cells (including egg cells), disappears after fertilization before the zygotic cell division, and is detected again when the sporophyte apical stem cell stops dividing. Sporophyte apical stem cells continuously divide in a *clf* deletion mutant, and induction of Physcomitrella *CLF* in the mutant stops this division [42]. CLF interacts with another PRC2 component, FIE, suggesting that PRC2 functions in this process as a complex [43]. The dominance of the sporophyte compared to the gametophyte is one of the most conspicuous innovations of land plant evolution [44]. This evolutionary process would have required the acquisition of long-lasting sporophyte stem cells, and comparisons of the PRC2 regulatory systems between flowering plants and Physcomitrella may provide a clue to elucidate the changes in regulatory networks responsible for the evolution of the different body plans.

WUSCHEL (*WUS*), *CLAVATA1* (*CLV1*), *CLV3*, and their related genes function in shoot and root meristems in angiosperm sporophytes [45]. However, the Physcomitrella genome does not contain putative orthologs of *WUS* and *CLV1* genes, although their homologs with unknown functions were found [46,47]. These angiosperm genes function in cellular interactions between stem cells and niche cells. Characterization of related genes in Physcomitrella may shed light on niche cells, which have not been recognized in non-vascular plants. Class 1 KNOX genes are another indispensable factor for shoot stem cell initiation and maintenance in angiosperms [48] with regulation at the upper levels [49,50]. However, loss of class 1 KNOX genes in Physcomitrella does not cause defects in sporophyte stem cell division other than slight changes in the division angle, but instead causes pleiotropic defects in the later stages of development, suggesting that meristem functions of class 1 KNOX genes were acquired

in the angiosperm lineage after the divergence of the moss lineage [51].

After the PRC2-mediated arrest of sporophyte apical stem cell activity, the cells that have already been produced divide to form the three major parts of a sporophyte body: a sporangium that forms spores via meiosis, a seta that serves as a stalk for the sporangium, and a foot that anchors the sporophyte and interacts with the gametophore tissue [39,51]. The seta meristem has higher cell division activity than does the surrounding tissue but no cells with stem cell characteristics have been described.

Switches to form gametophyte and sporophyte stem cells

Apospory and apogamy are asexual reproductive modes in non-seed plants [52,53]. In apospory, gametophyte apical stem cells are formed from sporophyte cells without meiosis. In apogamy, sporophyte apical stem cells are formed from gametophyte cells without fertilization. Deletion mutants of *CLF* and *FIE* of the PRC2 form apical stem cells similar to those in sporophytes from the side branch initial cells of protonemata. These results, together with the *CLF* and *FIE* expression patterns, indicate that PRC2 represses the initiation of sporophyte apical stem cells in the gametophyte generation, likely acting via histone H3 K27me3 modification [42,43]. On the other hand, deletion mutants of class 2 KNOX genes form chloronema apical stem cells from young sporophyte cells without undergoing meiosis, indicating that these transcription factors repress the initiation of gametophyte stem cells in the sporophyte generation before meiosis and spore germination [54]. Other than these genes, no regulatory factors for apospory and apogamy have been reported in Physcomitrella, and regulatory networks of stem cell formation linked to meiosis and fertilization remain to be revealed.

Adventitious formation of stem cells from differentiated cells

In addition to the reprogramming of differentiated protonema cells to form apical stem cells via side branch initials during regular development, moss gametophyte cells become chloronema apical stem cells upon wounding [53]. Differently from seed plants, no callus is formed before stem cell formation and differentiated cells directly transform to produce stem cells. When a Physcomitrella gametophore leaf is excised, leaf cells facing the cut divide to produce chloronema apical stem cells in 48 hours, without any exogenous phytohormones [55]. Along with factors for cell cycle reentry, genes

involved in intrinsic auxin and cytokinin regulatory systems change in expression, as do other transcriptional regulators [56,57].

Future prospects

One of the merits of using Physcomitrella for plant stem cell research is the feasibility of continuous observations of stem cells at the cellular level because of Physcomitrella's simple body organization compared to that of angiosperms. Furthermore, differentiated cells in Physcomitrella are more easily reprogrammed to become stem cells, and the reprogramming process during stem cell formation is traceable under microscope [55]. As reviewed here, key factors to initiate and maintain each type of stem cells and to switch between different types of stem cells during development have been reported in recent years. Future studies on their interacting factors as well as identification of new factors should provide more insights into stem cell biology.

Different types of stem cells evolved in multicellular organisms, resulting in the diversity of body plans and life cycles. However, evolutionary relationships of gene regulatory networks (GRNs) between different types of stem cells have not been well studied, and it is still unknown whether they originate from a common ancestral GRN or evolved independently, and how they diversified. Physcomitrella has at least eight types of stem cells and comparisons of each GRN will be useful to deduce the evolution of stem cell GRNs in relation to the life cycle. To date, comparisons of GRNs in different types of stem cells have revealed that auxin functions in protonema [19], gametophore [29], and sporophyte [58] apical stem cells, but other common factors have not been characterized. Analyses of auxin regulation and signaling pathways as well as transport in different types of stem cells may help to elucidate evolutionary processes in stem cell regulation.

Extant green algae that are sister to land plants have a haplontic life cycle and form only haploid stem cells [59]. This indicates that GRNs of gametophyte stem cells preceded those of sporophyte stem cells. Comparisons of GRNs between gametophyte and sporophyte stem cells in Physcomitrella and between those of sporophyte stem cells in Physcomitrella and flowering plants should provide clues to understand the evolution of a new generation. Recent studies suggest that several GRNs from gametophytes were co-opted in sporophyte stem cells [23,29,35], while some GRNs evolved *de novo*

specifically in the sporophyte generation [41,51,58]. Further comparisons of stem cell regulators between Arabidopsis and Physcomitrella and other land plants including lycopods and monilophytes, located phylogenetically between angiosperms and mosses, as well as liverworts and hornworts will be useful to clarify the general features of their evolution. The change in dominance from gametophyte to sporophyte body is one a major characteristic of the evolution of land plants, and analyses of factors involved in the switching between generations, including PRC2 and class 2 KNOX genes, in other land plants will be informative. The next decades are poised to be an exciting period in which the evolutionary processes of stem cell regulation in land plants are revealed.

Acknowledgements

We appreciate Tsuyoshi Aoyama, Masaki Ishikawa, Tomoaki Nishiyama, Keiko Sakakibara, and Yosuke Tamada for constructive comments on this manuscript. Dr. Tsuyoshi Aoyama kindly provided photographs of side-branching cells. We apologize to colleagues whose work we could not cite due to space limitations. The lab of Mitsuyasu Hasebe is funded through JST, JSPS, and MEXT.

References

1. Sparks EE, Imai A, Hasebe M, Benfey PN: **Developmental regulation and *de novo* formation of stem cells in plants**. In *Stem Cells: From Basic Research to Therapy*. Edited by Calegari F, Waskow C: Science Publishers; 2013. vol 1. Basic Stem Cell Biology, Tissue Formation during Development, and Model.

** The authors comprehensively reviewed molecular mechanisms of stem cell formation and maintenance during development as well as reprogramming from differentiated cells to stem cells in Arabidopsis and Physcomitrella. They stressed how plants provide a tractable experimetnal system for stem cell research.

2. Birnbaum KD, Sánchez Alvarado A: **Slicing across kingdoms: regeneration in plants and animals**. *Cell* 2008, **132**:697-710.
3. Schneider H, Pryer KM, Cranfill R, Smith AR, Wolf PG: **Evolution of vascular plant body plans: a phylogenetic perspective.** In *Developmental Genetics and Plant Evolution*. Edited by Cronk QC, Bateman RM, Hawkins JA: Taylor & Francis; 2002:330-364.
4. Steeves TA, Sussex IM: *Patterns in Plant Development* edn 2nd. Cambridge: Cambridge Univ. Press; 1989.
5. Kubo M, Imai A, Nishiyama T, Ishikawa M, Sato Y, Kurata T, Hiwatashi Y, Reski R, Hasebe M: **System for stable β-estradiol-inducible gene expression in the moss *Physcomitrella patens***. *PLoS One* 2013, **8:**e77356.
6. Schaefer DG, Zrÿd J-P: **Efficient gene targeting in the moss *Physcomitrella patens***. *Plant J* 1997, **11**:1195-1206.
7. Zimmer AD, Lang D, Buchta K, Rombauts S, Nishiyama T, Hasebe M, Van de Peer Y, Rensing SA, Reski R: **Reannotation and extended community resources for the genome of the non-seed plant *Physcomitrella patens* provide insights into the evolution of plant gene structures and functions**. *BMC Genomics* 2013, **14**:498.
8. Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, et al.: **The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants**. *Science* 2008, **319**:64-69.
9. Menand B, Calder G, Dolan L: **Both chloronemal and caulonemal cells expand by tip growth in the moss *Physcomitrella patens***. *J Exp Bot* 2007, **58**:1843-1849.

10. Jenkins GI, Cove DJ: **Light requirements for regeneration of protoplasts of the moss *Physcomitrella patens***. *Planta* 1983, **157**:39-45.
11. Xiao L, Zhang L, Yang G, Zhu H, He Y: **Transcriptome of protoplasts reprogrammed into stem cells in *Physcomitrella patens***. *PLoS One* 2012, **7**:e35961.
12. Cove D: **The moss *Physcomitrella patens***. *Annu Rev Genet* 2005, **39**:339-358.
13. Sugiyama T, Ishida T, Tabei N, Shigyo M, Konishi M, Yoneyama T, Yanagisawa S: **Involvement of PpDof1 transcriptional repressor in the nutrient condition-dependent growth control of protonemal filaments in *Physcomitrella patens***. *J Exp Bot* 2012, **63**:3185-3197.
14. Olsson T, Thelander M, Ronne H: **A novel type of chloroplast stromal hexokinase is the major glucose-phosphorylating enzyme in the moss *Physcomitrella patens***. *J Biol Chem* 2003, **278**:44439-44447.
15. Thelander M, Olsson T, Ronne H: **Effect of the energy supply on filamentous growth and development in *Physcomitrella patens***. *J Exp Bot* 2005, **56**:653-662.
16. Imaizumi T, Kadota A, Hasebe M, Wada M: **Cryptochrome light signals control development to suppress auxin sensitivity in the moss *Physcomitrella patens*.** *Plant Cell* 2002, **14**:373-386.
17. Kadota A, Sato Y, Wada M: **Intracellular chloroplast photorelocation in the moss *Physcomitrella patens* is mediated by phytochrome as well as by a blue-light receptor**. *Planta* 2000, **210**:932-937.
18. Ashton NW, Grimsley NH, Cove DJ: **Analysis of gametophytic development in the moss, *Physcomitrella patens,* using auxin and cytokinin resistant mutants.** *Planta* 1979, **144**:427-435.
19. Jang G, Dolan L: **Auxin promotes the transition from chloronema to caulonema in moss protonema by positively regulating *PpRSL1* and *PpRSL2* in *Physcomitrella patens***. *New Phytol* 2011, **192**:319-327.
20. Hayashi K, Horie K, Hiwatashi Y, Kawaide H, Yamaguchi S, Hanada A, Nakashima T, Nakajima M, Mander LN, Yamane H, et al.: **Endogenous diterpenes derived from ent-kaurene, a common gibberellin precursor, regulate protonema differentiation of the moss *Physcomitrella patens***. *Plant Physiol* 2010, **153**:1085-1097.

21. Lavy M, Prigge MJ, Tigyi K, Estelle M: **The cyclophilin DIAGEOTROPICA has a conserved role in auxin signaling**. *Development* 2012, **139**:1115-1124.

*Cyclophilin belongs to a family of peptidyl-prolyl cis-trans isomerase proteins catalyzing the cis-trans isomerization of peptide bonds preceding proline residues and involved in auxin signaling in flowering plants. The authors showed the cyclophilin-encoding gene *PpDGT* is involved in auxin signaling in Physcomitrella. Together with their previous study on the conserved TIR/AFB-dependent auxin signaling cascade (see 22), this showed that auxin perception pathways are mostly conserved in land plants.

22. Prigge MJ, Lavy M, Ashton NW, Estelle M: ***Physcomitrella patens* auxin-resistant mutants affect conserved elements of an auxin-signaling pathway**. *Curr Biol* 2010, **20**:1907-1912.

23. Pires ND, Yi K, Breuninger H, Catarino B, Menand B, Dolan L: **Recruitment and remodeling of an ancient gene regulatory network during land plant evolution**. *Proc Natl Acad Sci U S A* 2013, **110**:9571-9576.

**The authors compared gene regulatory networks of ROOT HAIR DEFECTIVE SIX-LIKE (RSL) transcription factors and auxin during caulonema differentiation in Physcomitrella and root hair formation in Arabidopsis. They found RSL-auxin subnetworks were remodeled in both lineages from the last common ancestor and are more hierarchical in Arabidopsis.

24. Cove DJ, Knight CD: **The moss *Physcomitrella patens*, a model system with potential for the study of plant reproduction.** *Plant Cell* 1993, **5**:1483-1488.

25. Harrison CJ, Roeder AH, Meyerowitz EM, Langdale JA: **Local cues and asymmetric cell divisions underpin body plan transitions in the moss *Physcomitrella patens***. *Curr Biol* 2009, **19**:461-471.

26. Doonan JH, Cove, D.J., Corke, F.M.K. and Lloyd, C.W.: **Pre-prophase band of microtubules, absent from tip-growing moss filaments, arises in leafy shoots during transition to intercalary growth**. *Cell Motil Cytoskel* 1987, **7**:138-153.

27. Spinner L, Pastuglia M, Belcram K, Pegoraro M, Goussot M, Bouchez D, Schaefer DG: **The function of TONNEAU1 in moss reveals ancient mechanisms of division plane specification and cell elongation in land plants**. *Development* 2010, **137**:2733-2742.

28. Vivancos J, Spinner L, Mazubert C, Charlot F, Paquet N, Thareau V, Dron M,

Nogue F, Charon C: **The function of the RNA-binding protein TEL1 in moss reveals ancient regulatory mechanisms of shoot development**. *Plant Mol Biol* 2012, **78**:323-336.

29. Aoyama T, Hiwatashi Y, Shigyo M, Kofuji R, Kubo M, Ito M, Hasebe M: **AP2-type transcription factors determine stem cell identity in the moss *Physcomitrella patens***. *Development* 2012, **139**:3120-3129.
30. Cove D, Bezanilla M, Harries P, Quatrano R: **Mosses as model systems for the study of metabolism and development**. *Annu Rev Plant Biol* 2006, **57**:497-520.
31. Saleh O, Issman N, Seumel GI, Stav R, Samach A, Reski R, Frank W, Arazi T: ***MicroRNA534a* control of *BLADE-ON-PETIOLE 1* and *2* mediates juvenile-to-adult gametophyte transition in *Physcomitrella patens***. *Plant J* 2011, **65**:661-674.
32. Duckett JG, Schmid AM, Ligrone R: **Protonemal morphogenesis**. In *Bryology for the Twenty-first Century*. Edited by Bates JW, Ashton NW, Duckett JG: British Bryological Society; 1998:223-246.
33. Sakakibara K, Nishiyama T, Sumikawa N, Kofuji R, Murata T, Hasebe M: **Involvement of auxin and a homeodomain-leucine zipper I gene in rhizoid development of the moss *Physcomitrella patens*.** *Development* 2003, **130**:4835-4846.
34. Jang G, Yi K, Pires ND, Menand B, Dolan L: **RSL genes are sufficient for rhizoid system development in early diverging land plants**. *Development* 2011, **138**:2273-2281.
35. Menand B, Yi K, Jouannic S, Hoffmann L, Ryan E, Linstead P, Schaefer DG, Dolan L: **An ancient mechanism controls the development of cells with a rooting function in land plants.** *Science* 2007, **316**:1477-1480.
36. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, Beeckman T, Kepinski S, Traas J, Bennett MJ, et al.: **A novel sensor to map auxin response and distribution at high spatio-temporal resolution**. *Nature* 2012, **482**:103-106.
37. Hohe A, Rensing SA, Mildner M, Lang D, Reski R: **Day length and temperature strongly influence sexual reproduction and expression of a novel MADS-box gene in the moss *Physcomitrella patens*.** *Plant Biol* 2002,

4:595-602.

38. Landberg K, Pederson ER, Viaene T, Bozorg B, Friml J, Jonsson H, Thelander M, Sundberg E: **The moss *Physcomitrella patens* reproductive organ development is highly organized, affected by the two *SHI*/*STY* genes and by the level of active auxin in the *SHI*/*STY* expression domain**. *Plant Physiol* 2013, 162:1406-1419.

*The authors described the detailed processes of archegonium and antheridium development and found that these organs are formed centrifugally to the first one. These organs retain apical stem cells, and the mechanism for successive formation of plural apical stem cells is an interesting topic for future tstudy.

39. Kofuji R, Yoshimura T, Inoue H, Sakakibara K, Hiwatashi Y, Kurata T, Aoyama T, Ueda K, Hasebe M: **Gametangia development in the moss *Physcomitrella patens***. In *Annual Plant Reviews, Vol 36: The moss Physcomitrella patens*. Edited by Cove D, Perroud F, Knight C: Wiley-Blackwell; 2009:167-181.

40. Tanahashi T, Sumikawa N, Kato M, Hasebe M: **Diversification of gene function: homologs of the floral regulator *FLO*/*LFY* control the first zygotic cell division in the moss *Physcomitrella patens*.** *Development* 2005, **132**:1727-1736.

41. Maizel A, Busch MA, Tanahashi T, Perkovic J, Kato M, Hasebe M, Weigel D: **The floral regulator *LEAFY* evolves by substitutions in the DNA binding domain.** *Science* 2005, **308**:260-263.

42. Okano Y, Aono N, Hiwatashi Y, Murata T, Nishiyama T, Ishikawa T, Kubo M, Hasebe M: **A polycomb repressive complex 2 gene regulates apogamy and gives evolutionary insights into early land plant evolution**. *Proc Natl Acad Sci USA* 2009, **106**:16321-16326.

43. Mosquna A, Katz A, Decker EL, Rensing SA, Reski R, Ohad N: **Regulation of stem cell maintenance by the Polycomb protein FIE has been conserved during land plant evolution**. *Development* 2009, **136**:2433-2444.

44. Kenrick P, Crane PR: **The origin and early evolution of plants on land.** *Nature* 1997, **389**:33-39.

45. Kiyohara S, Sawa S: **CLE signaling systems during plant development and nematode infection**. *Plant Cell Physiol* 2012, **53**:1989-1999.

46. Banks JA, Nishiyama T, Hasebe M, Bowman JL, Gribskov M, dePamphilis C,

Albert VA, Aono N, Aoyama T, Ambrose BA, et al.: **The Selaginella genome identifies genetic changes associated with the evolution of vascular plants**. *Science* 2011, **332**:960-963.

** The authors reported the first lycopod genome of *Selaginella moellendorffii* to be sequenced. Comparisons and phylogenetic analyses of genes related to stem cell formation and maintenance in Arabidopsis and their orthologs in *S. moellendorffii*, Physcomitrella, and green algae revealed extensive changes of the number of family members in each lineage and the lack of orthologs in both lycopod and moss genomes or either genome.

47. van der Graaff E, Laux T, Rensing SA: **The WUS homeobox-containing (WOX) protein family**. *Genome Biol* 2009, **10**:248.

48. Hay A, Tsiantis M: **KNOX genes: versatile regulators of plant development and diversity**. *Development* 2010, **137**:3153-3165.

49. Bolduc N, Yilmaz A, Mejia-Guerra MK, Morohashi K, O'Connor D, Grotewold E, Hake S: **Unraveling the KNOTTED1 regulatory network in maize meristems**. *Genes Dev* 2012, **26**:1685-1690.

50. Jasinski S, Piazza P, Craft J, Hay A, Woolley L, Rieu I, Phillips A, Hedden P, Tsiantis M: **KNOX action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities.** *Curr Biol* 2005, **15**:1560-1565.

51. Sakakibara K, Nishiyama T, Deguchi H, Hasebe M: **Class 1 KNOX genes are not involved in shoot development in the moss *Physcomitrella patens* but do function in sporophyte development**. *Evol Dev* 2008, **10**:555-566.

52. Raghavan V: *Developmental Biology of Fern Gametophytes*. Cambridge: Cambridge Univ. Press; 1989.

53. Chopra RN, Kumra PK: *Biology of Bryophytes*. New Delhi: Wiley Eastern Ltd.; 1988.

54. Sakakibara K, Ando S, Yip HK, Tamada Y, Hiwatashi Y, Murata T, Deguchi H, Hasebe M, Bowman JL: **KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants**. *Science* 2013, **339**:1067-1070.

** Functions of class 2 KNOX homeobox genes are still unknown in Arabidopsis. The authors found that Physcomitrella class 2 KNOX genes repress sporophyte cells to confer chloronema apical cell fate, indicating that these genes are key

regulators in the switch from the sporophyte generation to the haploid generation.

55. Ishikawa M, Murata T, Sato Y, Nishiyama T, Hiwatashi Y, Imai A, Kimura M, Sugimoto N, Akita A, Oguri Y, et al.: **Physcomitrella cyclin dependent kinase A links cell cycle reactivation to other cellular changes during reprogramming of leaf cells**. *Plant Cell* 2011, **23**:2924-2938.

** The authors established a new experimental system in which to analyze reprogramming of differentiated cells to stem cells using excised gametophore leaves in Physcomitrella. They characterized the reprogramming process and reported that the cell cycle is arrested at the late S-phase in the gametophore leaf cells and cyclin dependent kinase A regulates acquisition of tip growth as well as cell cycle reentry.

56. Nishiyama T, Miyawaki K, Ohshima M, Thompson K, Nagashima A, Hasebe M, Kurata T: **Digital gene expression profiling by 5'-end sequencing of cDNAs during reprogramming in the moss *Physcomitrella patens***. *PLoS ONE* 2012, **7**:e36471.

57. Busch H, Boerries M, Bao J, Hanke ST, Hiss M, Tiko T, Rensing SA: **Network theory inspired analysis of time-resolved expression data reveals key players guiding *P. patens* stem cell development**. *PLoS One* 2013, **8**:e60494.

58. Fujita T, Sakaguchi H, Hiwatashi Y, Wagstaff SJ, Ito M, Deguchi H, Sato T, Hasebe M: **Convergent evolution of shoots in land plants: lack of auxin polar transport in moss shoots**. *Evol Dev* 2008, **10**:176-186.

59. Graham LE, Graham JE, Wilcox LW: *Algae* edn 2nd. Upper Saddle River, New Jersey: Prentice Hall, Inc.; 2008.

Figure legends

Figure 1. Stem cells formed in the life cycle of Physcomitrella. Eight types of stem cells are numbered on arrowheads corresponding to Box 1. (a) Spore. (b) Germinated spore with a chloronema apical stem cell (arrowhead). (c) Chloronema filament with a chloronema apical stem cell (arrowhead) and produced chloronema cells. Cell septa are indicated by arrows. (d) Chloronema apical cell with a rectangular septum and round, green chloroplasts. (e) Chloronema side branch initial cell formed on a chloronema cell to become a secondary chloronema apical stem cell. (f) Caulonema filament with a caulonema apical stem cell (arrowhead) and produced caulonema cells. Cell septa are indicated by arrows. (g) Caulonema apical stem cell with an oblique septum and spindle-shaped, less-green chloroplasts. (h) Side branch initial cell protruding from a caulonema cell (arrow). (i) Secondary chloronema apical stem cell transformed from a side branch initial cell (arrowhead). (j) Swelled gametophore apical stem cell transformed from a side branch initial cell (arrowhead). (k) Young gametophore with a gametophore apical stem cell (arrowhead) at the tip. (l) Gametophore with rhizoids. (m) Longitudinal optical section of a gametophore tip showing a gametophore apical stem cell (white arrowhead) and leaf apical stem cells (yellow arrowheads). (n) Longitudinal optical section of a leaf primordium showing a leaf apical stem cell (arrowhead). (o) Gametophore leaf with a mid-vein. (p) Rhizoid initiated from a gametophore epidermal cell. (q) Rhizoid with a rhizoid apical stem cell (arrowhead). (r) Longitudinal optical section of an antheridium apical stem cell (arrowhead). (s) Growing antheridia and antheridium apical stem cells (arrowheads). (t) Longitudinal optical section of an archegonium apical stem cell (arrowhead). (u) Growing archegonium in which activity of an archegonium apical stem cell is lost. (v) Matured archegonia and antheridia. (w) Twelve cell-stage sporophyte with a sporophyte apical stem cell (arrowhead). (x) Immature sporophyte isolated from a gametophore. (y) Gametophore with a sporophyte (bracket) at the tip. (m), (n), (r) and (t) were stained with calcofluor. Calcofluor fluorescence was excited at 405 nm with a blue diode laser and detected with a LP 420 filter. Bars = 20 μm in (a), (b), (k), (m), (n), (r)-(u), (w); 100 μm in (c)-(j), (q), (v), (x); 500 μm in (o), (p); and 1 mm in (l), (y). Pictures in (h) to (k) were kindly provided by Dr. Tsuyoshi Aoyama.

Figure 2. Schematic diagrams of stem cell formation. Formation processes of a gametophore apical stem cell (a), leaf apical stem cells (b, c), an antheridium apical stem cell (d), an archegonium apical stem cell (e), a sporophyte apical stem cell (f). Apical stem cells are colored as follows: gametophore, yellow; leaf, light blue; antheridium, orange; archegonium, pink; and sporophyte, green.

Table 1. Diversity of stem cells in land plants.

	Sporophyte		Gametophyte	
	Stem cells	Niche	Stem cells	Niche
Angiosperms	Multiple cells (shoot, root, cambium)	Multiple cells (shoot, root); unknown (cambium)	No	No
Gymnosperms	Multiple cells (shoot, root); Unknown (cambium)	Unknown	No	No
Monilophytes (except Leptosporangiate ferns)	Single cell (shoot, root)	Unknown	Single cell (thallus)	Unknown
Monilophytes (Leptosporangiate ferns)	Single cell (shoot, root, sporangium)	Unknown	Single cell (thallus, gametangium)	Unknown
Lycopods (Lycopodiaceae, Isoetaceae)	Multiple cells (shoot, root)	Unknown	Unknown	Unknown
Lycopods (Selaginellaceae)	Single cell (shoot, root)	Unknown	Unknown	Unknown
Bryophytes (Mosses)	Single cell (sporophyte)	Unknown	Single cell (protonema, gametophore, leaf, rhizoid, gametangium)	Unknown
Bryophytes (Hornworts)	Unknown	Unknown	Single (protonema, thallus, gametangium)	Unknown
Bryophytes (Liverworts)	No	No	Single (protonema, thallus, rhizoid, gametangium)	Unknown

Box 1. Eight types of stem cells in Physcomitrella. Numbers corresponds to those in Figure 1.

Stem cells in the haploid generation

1. Chloronama apical stem cell
2. Caulonema apical stem cell
3. Gametophore apical stem cell
4. Leaf apical stem cell
5. Rhizoid apical stem cell
6. Antheridium apical stem cell
7. Archegonium apical stem cell

Stem cell in the diploid generation

8. Sporophyte apical stem cell

Figure 1 Kofuji and Hasebe

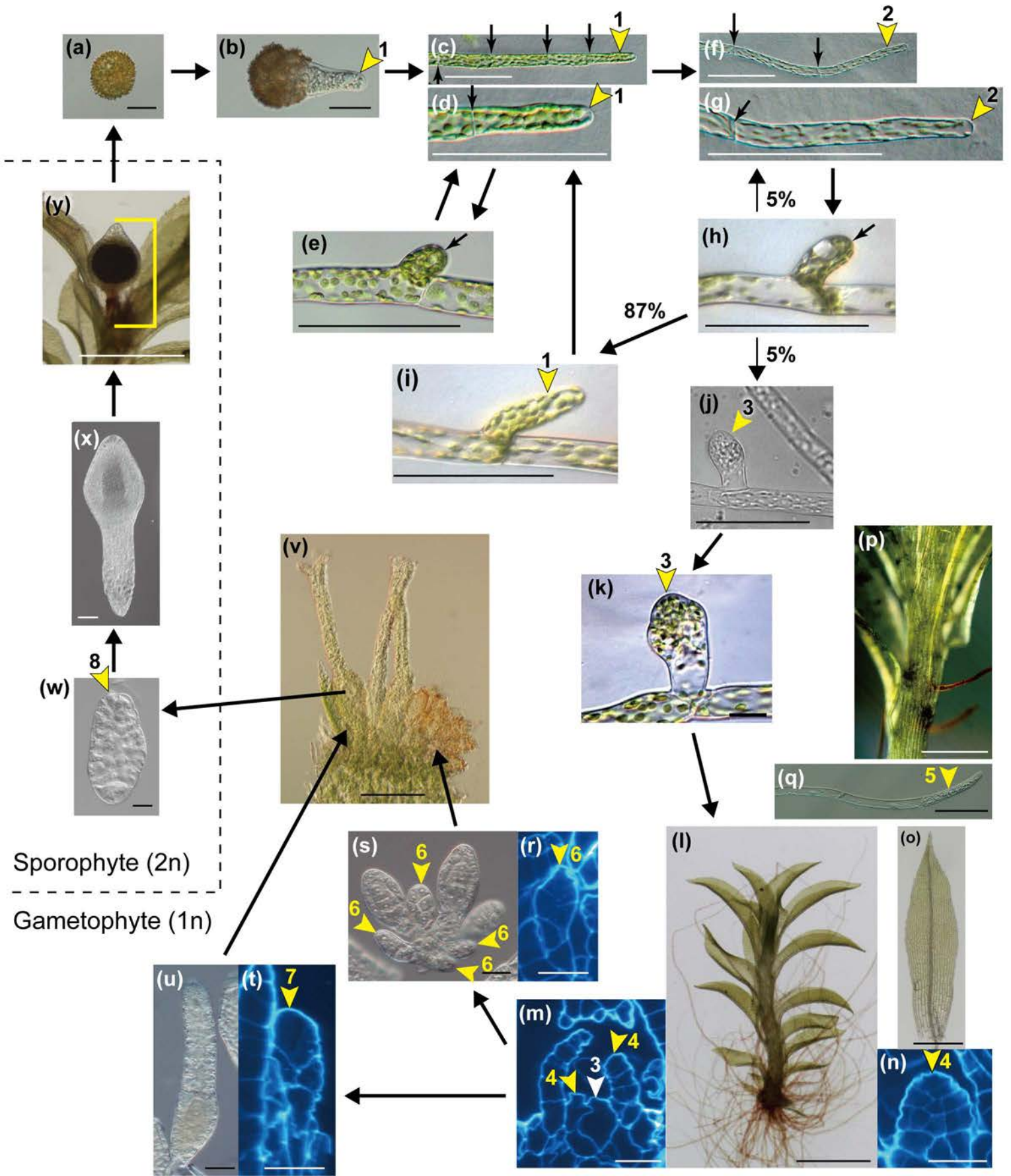

Figure 2 Kofuji and Hasebe